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US	60/150,761 (CIP)
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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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Published:
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18 October 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/14518 A3

(54) Title: METHODS FOR GENERATING DOUBLED HAPLOID PLANTS

(57) Abstract: The present invention provides methods for generating doubled haploid and/or haploid plants from microspores. The methods of the invention include the steps of (a) purifying microspores at a developmental stage amenable to androgenic induction; (b) subjecting said microspores to nutrient stress to obtain stressed microspores; (c) contacting said microspores with an amount of a sporophytic development inducer effective to induce sporophytic development, said contacting step occurring before, during, after, or overlapping with any portion of said nutrient stress step; and (d) culturing said isolated microspores with at least one live plant ovary or with an aliquot of plant ovary conditioned medium. The methods of the present invention can also include the step of genetically transforming the microspores to produce genetically transformed plants.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/18790

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12N 5/00, 15/05, 15/82; A01H 1/08, 4/00, 5/00
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/260, 278, 299, 320, 320.3; 435/468, 410, 419, 420, 430.1, 431, 242

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,272,072 A (KANEKO et al) 21 December 1993, abstract	43
—		—
Y		43
Y	KASHA, K.J. et al. Haploids in Cereal Improvement: Anther and Microspore Culture. Gene Manipulation in Plant Improvement II. 1990, pages 213-230, especially pages 219 and 224-227.	1-45
Y	US 5,322,789 A (GENOVESI et al) 21 June 1994, col. 4, lines 18-21, 25-27, 41-48, 60-63, 67-68, col. 5, lines 5-8, col. 6, 1-40, col. 16, lines 19-63, col. 23, lines 32 and 36.	1-45

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 27 MARCH 2001	Date of mailing of the international search report 01 MAY 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ANNE MARIE GRUNBERG Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/18790

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KOHLER, F. et al. Regeneration of Isolated Barley Microspores in Conditioned Media and Trials to Characterize the Responsible Factor. J. Plant Physiol. 1985, Vol. 121, pages 181-191, especially page 181.	1-45

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/18790

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

800/260, 278, 299, 320, 320.3; 435/468, 410, 419, 420, 430.1, 431, 242

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: BARRY F. MCGURL
CHRISTENSEN O'CONNOR JOHNSON
KINDNESS PLLC
1420 FIFTH AVE., SUITE 2800
SEATTLE, WA 98101

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	05 MAY 2003

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
KONC-115968	

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US00/18790	23 AUGUST 2000	26 AUGUST 1999

Applicant
NORTHWEST PLANT BREEDING CO.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	ANNE MARIE GRUNBERG
Facsimile No. (703) 305-3230	Telephone No. (703) 308-6196

Form PCT/IPEA/416 (July 1992)★

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference KONC-115968	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/18790	International filing date *(day/month/year)* 23 AUGUST 2000	Priority date *(day/month/year)* 26 AUGUST 1999
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant NORTHWEST PLANT BREEDING CO.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of — sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☒ Certain defects in the international application

   VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07 MARCH 2001	30 APRIL 2003
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer ANNE MARIE GRUNBERG
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/18790

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-37 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the claims:
pages 38-43 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the drawings:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the sequence listing part of the description: NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets~~/fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/18790

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	1-42 and 44-45	YES
	Claims	43	NO
Inventive Step (IS)	Claims	none	YES
	Claims	1-45	NO
Industrial Applicability (IA)	Claims	1-45	YES
	Claims	none	NO

2. citations and explanations (Rule 70.7)

Claim 43 lacks novelty under PCT Article 33(2) as being anticipated by Kaneko et al.

Claim 43 is drawn to genetically transformed plants which may be either haploid or diploid in nature (due to spontaneous doubling of chromosomes).

Kaneko et al teach genetically transformed plants (first sentence of the abstract). The growth process taught by Kaneko et al differs from the claimed growth process in that a sporophytic development inducer was not used to induce sporophytic development. However, the claimed method of producing transgenic plants from microspores using a sporophytic development inducer would not distinguish the transgenic plant itself from that taught by Kaneko et al. The process of making the product fails to distinguish the two products. Thus, the claimed invention was clearly *prima facie* obvious as a whole to one of ordinary skill in the art, if not anticipated by Kaneko et al.

Claims 1-45 lack an inventive step under PCT Article 33(3) as being obvious over Kasha et al.

Claims 1-45 are drawn to a method of producing plants from microspores wherein microspores are selected at a developmental stage amenable to androgenic induction. The microspores are subjected to temperature stress and a sporophytic development inducer, after which they are isolated and cocultured with either ovary-conditioned medium or at least one live plant ovary. Microspores may also be starvation stressed and the medium may contain an auxin, a cytokinin, or gibberellin. A cell spindle inhibiting agent may be used to induce diploidy. Microspores may also be genetically transformed.

Kasha et al is a review article summarizing the state of the art of anther and microspore culture in 1990. Kasha et al teach a method of producing plants from microspores comprising selecting plant material with microspores at a developmental stage amenable to androgenic induction (page 224, line 3 under "III. Microspore Culture"; subjecting the microspores to temperature stress to obtain stressed microspores (page 224, (Continued on Supplemental Sheet.)

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

Claim 26 is objected to under PCT Rule 66.2(a)(iii) as containing the following defect(s) in the form or contents thereof: "benzaminopurine" should be changed to --benzylaminopurine--.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 1, 43, and 45 are objected to as lacking clarity under PCT Rule 66.2(a)(v) because practice of the claimed invention is not enabled as required under PCT Rule 5.1(a) for the reasons set forth in the following paragraph.

Claims 1, 43, and 45, as well as their dependent claims are enabled if they are limited to wheat, and plant material comprising microspores. The description does not reasonably provide enablement for claims broadly drawn to all plant varieties or all plant material. The description does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The description only provides guidance for obtaining wheat plants produced from microspores. No guidance is provided regarding the production of any other plants from microspores or from any other plant material not comprising microspores. In contrast, the claims are broadly drawn to any plant variety and any plant material. However, by following the methodologies in the description, one skilled in the art would only have been able to produce wheat from microspores.

It is well known in the art that tissue culture conditions, media, and procedure differ depending on the type of plant, or even genotype. The description does not teach how any other plant except for wheat can be obtained from the procedure set forth. Additionally, the description does not teach how any other plant material can be used to obtain cultured plants.

Given the claim breadth, unpredictability, and lack of guidance as discussed above, undue experimentation would have been required by one skilled in the art to produce any type of plant from any plant material.

Claims 1 and 45 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): It is unclear in step (c) whether stressed or purified microspores are contacted. There is a lack of antecedent basis in step (b) for "said purified microspores" and in step (d) for "said isolated microspores". Additionally, the claims are incomplete because the steps do not result in the product specified in the preamble. The product in the preamble specifies a "plant", however the last step in the claims produces a microspore.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:
The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): C12N 5/00, 15/05, 15/82; A01H 1/08, 4/00, 5/00 and US Cl.: 800/260, 278, 299, 320, 320.3; 435/468, 410, 419, 420, 430.1, 431, 242

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
halfway through the first paragraph under "III. Microspore Culture"; page 225, bottom of second paragraph; page 227, lines 3-6); contacting the microspores with an amount of a sporophytic development inducer effective to induce sporophytic development (page 227, bottom and middle of the first paragraph); isolating the microspores (page 224, lines 3-8 under "III. Microspore Culture"); and coculturing the isolated microspores with either ovary-conditioned medium or at least one live plant ovary (page 224, first sentence of the second paragraph under "III. Microspore Culture"; page 227, secong paragraph). The microspores within the selected plant material are in the mid uninucleate to early binucleate stage of development (page 224, line 13 under "III. Microspore Culture"; page 225, line 8; page 225, first sentence in the second paragraph; page 226, first paragraph under "A. Pollen Embryogenesis"). Microspores can be subjected to temperature stress with either high or low temperatures (page 227, line 4). The sporophytic development inducer includes 2-chloroethylphosphonic acid or other chemical sterilants related to ethylene production (page 227, bottom of first paragraph). Auxins, such as 2,4-dichlorophenoxyacetic acid are widely used in tissue culture (page 219, 3rd paragraph). Genetic transformation is taught at page 225, first full paragraph.

Kasha et al do not teach starvation induced stress of microspores, nor particular stress temperatures or particular duration of temperature shock. Kasha et al also do not teach an aqueous medium comprising NPB 98 or NBP 99. Kasha et al do not teach the specific concentrations of sporophytic development inducer, auxins, cytokinins, or gibberellin. Kasha et al also do not teach contacting microspores with an effective amount of a cell spindle inhibiting agent, nor do they specifically teach stressed microspores isolated by density centrifugation, nor do they teach a solution of mannitol layered over a higher density solution of maltose.

It would have been *prima facie* obvious to a person of ordinary skill in the art at the time th einvention was made to utilize th emethod of producing plants from microspores as exemplified in the review article by Kasha et al. It is readily apparent that th evarious limitations were well known in the art. Particular temperatures for temperature stress applications, as well as time ranges, concentrations of auxins, cytokinins, gibberellins, media type and stressing procedures would have been an optimization of process parameters and would have depended upon the species and genotype. It was well known in the art at the time the invention was made that a cell spindle inhibiting agent, such as colchisine, could have been used to ensure doubled haploids. Density centrifugation was also widely used in the art for seperating microspores from other plant materials.

Claims 1-45 lack an inventive step under PCT Article 33(3) as being obvious over Genovesi et al in view of Kohler et al.

Genovesi et al teach a method of producing plants from microspores comprising selecting plant material with microspores at a developmental stage amenable to androgenic induction (column 4, lines 41-48, lines 60-63, for example); subjecting the microspores to temperature stress to obtain stressed microspores (column 4, lines 41-48, lines 60-63, for example); subjecting the microspores to temperature stress to obtain stressed microspores (column 4, lines 18-21, for example); contacting the microspores with an amount of a sporophtic development inducer effective to induce sporophytic development (column 16, lines 50-59); and isolating the microspores (column 4, lines 25-27, for example). The microspores within the selected plant material are preferably in the mid uninucleate stage of development (column 4, lines 67-68, for example). Microspores are subjected to temperature stress in a range of temperatures (column 4, lines 20-21; column 5, lines 5-8; page 227, line 4). The sporophytic development inducer is generically described at column 16, lines 49-60. Glycine, a sporophytic development inducer is described at column 16, line 63 of the specification. Auxin and cytokinin usgae is described at column 16, lines 19-47, and 2,4-dichlorophenoxyacetic acid is a well-known auxin widely used inthe art. Genovesi et al teach contacting microspores with an effective amount of cell spindle inhibiting agent (column 23, line 36, for example) and an effective amount of sporophytic development inducer (column 23, line 32, for instance). Additionally, at column 6, lines 1-40, various methods of microspore isolation are taught. Genovesi et al do not teach coculturing the isolated microspores with either ovary conditioned medium or at least one live plant ovary.

Kohler et al teach coculturing of isolated microspores with ovary-conditioned medium (page 181, summary, for example). It would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made to utilize the method of producing plants from microspores as taught by Genovesi et al, and to modify that method by coculturing isolated microspores with ovary-conditioned medium given the advantages of increased regeneration an dthe guarantee of cell divisions of the isolated microspores as described by Kohler et al in the summary on page 181.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

---------- NEW CITATIONS ----------
NONE

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 30 August 2001 (30.08.01)	
International application No. PCT/US00/18790	**Applicant's or agent's file reference** KONC-115968
International filing date (day/month/year) 23 August 2000 (23.08.00)	**Priority date** (day/month/year) 26 August 1999 (26.08.99)
Applicant KONZAK, Calvin, F. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

07 March 2001 (07.03.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

R. Forax

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